

Jeffrey MARLOW

Lewis and Clark Fund for Exploration and Field Research in Astrobiology

awarded May 31st, 2011

Project Report:

Carbonate Based Anaerobic Methane Oxidation at Hydrate Ridge Methane Seeps

The anaerobic oxidation of methane (AOM) is a unique extremophilic microbial metabolism that plays an important role in the global carbon cycle. The process, which is mediated by a consortium of two types of organisms – methane-consuming Archaea and sulfate-reducing Bacteria – consumes approximately 80% of the methane emerging from hydrocarbon seeps on the sea floor. Because of its role in methane sequestration, AOM is an important process in dampening global climate change, and its role will likely increase as methane deposits in high-latitude permafrost are mobilized with continued warming. This metabolism also serves as an analog for potential life beyond Earth, particularly on bodies with locally high levels of hydrocarbons. Despite the importance of AOM both for near-term climate control and in the search for life beyond Earth, little is known about how the participating organisms interact and how methane is processed biologically. This study addressed these key questions through genetic and proteomic analyses of sediment and carbonate rock samples, which provide a new window into how methane seeps are operating: which organisms are present and what they're doing.

Samples were collected during our *Atlantis* cruise to Hydrate Ridge (44° 40' N, 125° 06' W) between September 1st and 9th, 2011. After leaving the port of Astoria, Oregon, *Atlantis* floated over the methane seeps at Hydrate Ridge South, deploying the remotely operated vehicle (ROV) *JASON* to conduct reconnaissance and collect appropriate samples. In order to maximize sample collection, *JASON* remained on the seafloor, filling sample “elevators”, which were then sent to the surface and brought on-board. This productive arrangement allowed our expedition to set the record for longest *JASON* dive; the ROV was submerged during the entirety of our time at sea.

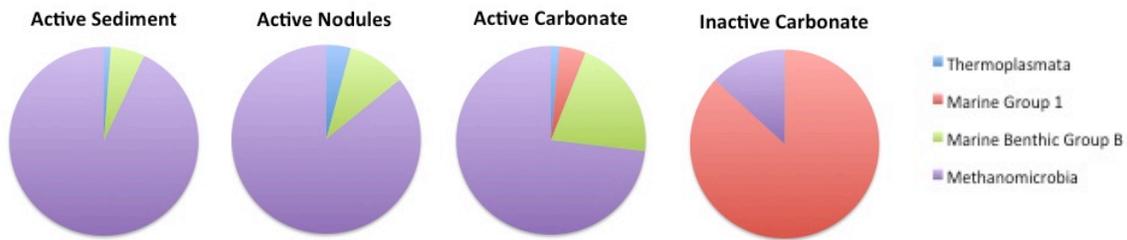
Previous work at Hydrate Ridge and other methane seeps had focused exclusively on the sediment at the seafloor. However, massive mounds of carbonate rock underlie this sediment, and this study hoped to determine if AOM-active microbial consortia could inhabit these environments. Establishing carbonate rock as a potential habitat would expand our understanding of the range of endolithic communities – organisms living inside rock pore spaces. Given the harsh external environments typical of most planetary surfaces (intense UV radiation, cold temperatures), endolithic environments are the most probable abode of any extraterrestrial microbes.

In order to determine the microbial activity of sediment, nodules (which are compacted, likely proto-lithic sediment masses), and carbonate rocks at both actively venting sites and previously active environments, 16s rRNA gene sequences were attained. By acquiring a few hundred full-length sequences, we can effectively determine where seep organisms fit on the tree of life and develop a quantitative sense of the species distributions. For the Archaea, class-level genetic diversity is shown below:

Jeffrey MARLOW

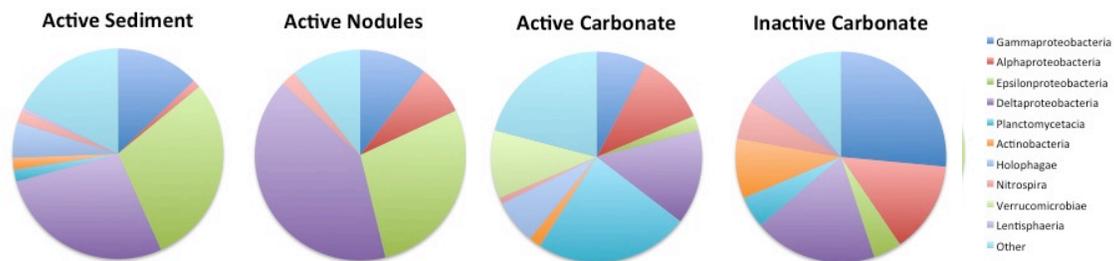
Lewis and Clark Fund for Exploration and Field Research in Astrobiology

awarded May 31st, 2011



This sequence information shows that materials within actively venting sites are broadly similar in terms of their Archaeal inhabitants. Methanomicria contain the “ANME” organisms, which are most frequently associated with methane oxidation, while the Marine Group I class is often considered a “background deep ocean” community. The dominance of Marine Group I in inactive carbonate rock samples therefore demonstrates that methane oxidizing organisms are supplanted as the flow of methane shuts off. Perhaps most intriguingly, the Methanomicria that do remain belong almost exclusively to the ANME-1 order. How these organisms persist without methane is unknown, but it appears to be an ability that other ANME organisms lack. It is possible that ANME-1, seeded by AOM activity, could persevere through decreases in methane levels and shift to methanogenesis, serving as a recorder of past methanotrophic activity throughout Earth’s history.

The Bacterial diversity reveals a more heterogeneous community structure, with 24 different classes represented among the four samples:



Deltaproteobacteria account for significant percentages of the clones from active sediment (27.4%) and nodules (41%); closely related cultured genera include sulfate reducers such as *Desulfobulbus*, *Desulfonema*, and *Desulfobacterium*. Within carbonates, Deltaproteobacteria are roughly half as prevalent, suggesting a smaller role for sulfate reducers. Even more pronounced is the dearth of Epsilonproteobacteria found in carbonates. The nearest cultured representatives to the Epsilonproteobacteria clones found in the active sediment and nodules are *Sulfurovum* and *Sulfurimonas* representatives, which are known sulfur oxidizers. These organisms likely utilize

Jeffrey MARLOW

Lewis and Clark Fund for Exploration and Field Research in Astrobiology

awarded May 31st, 2011

sulfides produced by sulfate reducing partners of AOM consortia; their near absence in the carbonates suggests that this secondary cycling of sulfur compounds is less active in the endolithic habitat.

The genetic analysis described above demonstrated that the carbonate rocks were active and genetically distinct from the associated sediments. The next step in characterizing the biological activity was to examine the proteins active in AOM conditions. While genes tell us which proteins *might* be synthesized, proteins demonstrate which enzymatic molecules are *actually* present, offering a more accurate perspective on biological functioning. Proteomic work is ongoing, but initial data sets indicate that many proteins needed for methane oxidation and sulfate reduction are present and produced at relatively high levels. Other, slightly less expected proteins such as transporters are also up-regulated in methane-rich conditions. Understanding why certain proteins are over-produced compared with others will tell us more about the molecular functioning of AOM.

Our continued progress with AOM systems will inform future studies of its relevance to astrobiological targets. For example, based on our discovery of endolithic AOM consortia, I am initiating a model-based study of potential endolithic AOM on Mars, where recent findings of methane plumes suggest that raw materials for methanotrophy are present. As we clarify which organisms and proteins are active in AOM on Earth, we can also narrow the catalog of biomarkers that could serve as evidence for such processes elsewhere.

The anaerobic oxidation of methane has been an important biological process throughout Earth's history, and it may prove relevant in the search for life beyond Earth. By studying the genetic and proteomic implications of modern AOM, the Lewis and Clark Fellowship has prepared me for further investigations into the past and future impacts of this important microbial process.



Jeffrey MARLOW

Lewis and Clark Fund for Exploration and Field Research in Astrobiology

awarded May 31st, 2011

The interior of the *JASON* control room.